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Master Degree in Veterinary Medicine

PERINATAL LAMB MORTALITY IN LIMOUSIN REGION

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Abstract

Background and objectives

This report analyses the lamb mortality in 57 sheep farms, with approximately 22.614 adult ewes (Mean ± SD: herd size=355.8 ± 255.7 ewes), in Haute-Vienne, a French department of the Limousin region, France. The lamb mortality rate in France was reported to be around 16% (Institut d'Élevage 2012).

The aim of this study was:

1) to determine the rate of perinatal lamb mortality in Limosin region
2) to identify the causes of lambs death and the trends and patterns of perinatal lamb mortality
3) to calculate the economic losses related to lamb mortality

Materials and methods

Ewe abortion and lamb mortality were observed and the evaluation of causes of death was studied by a cross-sectional study on 57 herds. The sample was selected using convenience sampling. Detailed necropsy and laboratory analyses were performed.

Results

The results of this study indicate that it was not possible to identify the ethiologic agent in most of the herds (59%). Due to the small positive agent sample size is impossible define the leading abortion causes.

The major risk factors identified for poor lamb survival were enterotoxaemia (25.5%), septicaemia (19.1%), colibacillosis (14.9%), coccidiosis (8.1%) and other various causes (31.9%).

The mortality was higher in lambs with 0-14 and 31-45 days old (36%) and the majority of lambs (55%) died without showing any clinical signs.

There was an average mortality rate, including abortions and lamb mortality, of 13.05% which represented a total loss of 116.706€/year to the 57 sheep farms.

Conclusion

The number of samples was insufficient to make any absolute inference about the leading causes of ewe abortions and lamb deaths but it was evident in this study that farmers are not aware of the importance of preventing the leading diseases. This may be due to an absence of a cost–benefit analysis.

Keywords: Abortion, Economic assessment, Ewe, Lamb, Lambing, Neonatal mortality

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To take a course is not the only result of my effort:
it is my team effort!

To my Parents

“This is only his box. The sheep you asked for is inside."

in Little Prince by Saint-Exupéry

List of abbreviations

- *C. burnetii*: *Coxiella burnetii*
- *E. coli*: *Escherichia coli*
- ml: mililitre
- *p*: p-value
- ®: registered trademark
- *S. bongori*: *Salmonella bongori*

List of acronyms

- CI: confidence interval
- CV: coefficient of variation
- DNA: Deoxyribonucleic acid
- ELISA: enzyme-linked immunosorbent assay
- EU: European Union
- ND: not defined
- OIE: World Organisation for Animal Health
- PCR: polymerase chain reaction
- SD: standard deviation
- TM: trademark
- UK: United Kingdom

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1. Introduction

During the internship of four months at the Veterinary Clinic *des Rochettes*, in the Limousin region, France, a study was done to analyse the problem of perinatal mortality in lambs. This study was carried out between January and April 2014.

Neonatal mortality is the cause of considerable economic losses and reduced animal welfare in sheep production (Mellor and Stafford 2004; Østerås *et al.* 2007). Some flocks experience severe losses, which may compromise the economic viability of a sheep farm (Holmoy *et al.* 2012).

The perinatal period in sheep extends from the 60th day after conception until the 28th day after birth (Dennis 1974; Rook *et al.* 1990; Haughey 1991; Bernal 2001). Due to the short period of this study, we decided to include lambs until 45 days old in order to increase its statistical strength.

As the main goal of sheep farmers in this region is the production of lambs, the reduction of perinatal morbidity and mortality is obviously pivotal for the farms.

1.1 Economic considerations

The ovine meat in France represents 10% of EU sheep meat sector being preceded by UK and Spain. Portugal merely produces 1.4% of total ovine meat produced at EU (Eurostat 2012) but it is relatively near of self-sufficiency with near 80% of the supply (Statistics Portugal 2012). France is not self sufficient as it depends on 45% of supply from UK, Ireland and New Zealand (Institut de l'Élevage & Confédération Nationale de l'Élevage 2013).

In France, agriculture and agri-food represent 5% of gross domestic product (Reussir Grandes Cultures 2013), while in Portugal, the gross domestic product of this area represents solely 3.48% (Espirito Santo Research 2013).

Overall, animal prices depend on supply and demand, which are in turn heavily influenced by season (Kassa *et al.* 2011) and the occurrence of religious and cultural festivals, drought and weather shocks (Gebre *et al.* 2012).

The economy of lamb meat production is conditioned to a large extent by the number of weaned lambs, which is directly related to both ewe fecundity and lamb survival (Madani *et al.* 2013).

According to a study by the Institut de l'Elevage in 2012, the annual ewe prolificacy in the Limousin region was 1.05 lambs/ewe/year and the average price of a lamb was 106 €, being 5.75€/Kg the average price of carcass.

1.2 Abortion in sheep

A low level of lamb losses from natural, uncontrollable causes is inevitable (Duncanson 2012). While sporadic losses are variably attributed to overcrowding, competition during feeding, handling procedures or movement, an abortion rate above 2% is suggestive of an infectious aetiology and laboratory investigation is strongly recommended. A standard set of samples allows laboratory identification of the abortifacient agent(s) in most situations. Farmers must isolate all suspect aborted sheep and remove all aborted material (Scott 2007). Most cases of abortion after the 120th day of pregnancy are usually caused by infectious diseases (Scott 2007).

The identification of an abortive agent can be often achieved through gross and histological evaluations of foetal and placental tissues, followed by routine microbiological analyses. However, two retrospective studies, consisting in tissue samples obtained from sheep and goat abortions and sent to two different diagnostic laboratories in the United States, demonstrated that it was not possible to identify a cause in 56% of the ovine abortions and 53% of the caprine abortions (Kirkbride 2012).

1.2.1 Ovine chlamydiosis

Ovine chlamydiosis, also known as enzootic abortion of ewe or ovine enzootic abortion, is caused by the bacteria *Chlamydophila abortus*. Typically Chlamydial abortion occurs in the last 2–3 weeks of pregnancy with the delivery of stillborn lambs and inflamed placentas. Infection can, however, also result in the delivery of full-term stillborn lambs or weak lambs that do not survive longer than 48 hours. There are rarely any predictive signs that abortion is going to occur (OIE 2013).

Pasture and the environment are contaminated by vaginal discharges, placenta and aborted foetuses, and an infected ewe can shed the organism for a week before aborting and for 2 weeks afterwards (Radostits *et al.* 2007).

Infection in aborting animals can be studied serologically by rising titers in paired serum samples and by culture of the organisms. Prophylactic measures include vaccination and isolation of aborting ewes (Radostits *et al.* 2007).

Chlamydiosis of small ruminants is a zoonosis and the organism must be handled with biosafety precautions. Pregnant women are particularly at risk (OIE 2013). The organism can be detected in sheep and cattle raw milk and, therefore, also can pose a risk for zoonotic infection (Radostits *et al.* 2007).

1.2.2 Q fever

Query (Q) fever (or Coxiellosis) is a zoonosis caused by *Coxiella burnetii*, a small Gram-negative bacteria, which is very resistant to physical and chemical agents and can survive in the environment and soil for several months (Radostits *et al.* 2007). This obligate intracellular bacteria displays different morphological forms in its developmental cycle. Some forms can survive in the environment due to a small, dense and highly resistant spore-like form (Coleman *et al.*, 2004; Heinzen *et al.* 1999).

In domestic ruminants, Q fever is mostly associated with sporadic abortions or outbreaks of abortions and dead or weak offspring, followed usually by recovery without complications (OIE 2013).

Infection in aborting animals can be studied serologically by fluorescent antibody staining and PCR. Prophylactic measures include vaccination and isolation of aborting ewe (Radostits *et al.* 2007).

C. burnetii is destroyed by pasteurization but there is a risk if e.g. members of a farm family consume raw milk (Radostits *et al.* 2007).

1.2.3 Salmonellosis

Salmonellosis is an infectious disease of humans and animals caused by organisms of two species of *Salmonella* (*Salmonella enterica*, and *S. bongori*). Although primarily intestinal, these rod-shaped and Gram-negative bacteria are widespread in the environment and are commonly found in farm effluents, human sewage and in any material subject to faecal contamination (OIE 2013). The organism persists in internal organs of the carriers for up to 6 months and it is excreted in the faeces and vaginal mucus for periods up to 4 months (Radostits *et al*. 2007).

Young, pregnant and lactating animals are the most susceptible. Enteric disease is the commonest clinical manifestation but a wide range of clinical signs, which include acute septicaemia, abortion, arthritis and respiratory disease, may be seen (OIE 2013).

Identification of the disease can be achieved by the isolation of the organism, which is present in large numbers in the foetus, placenta and uterine discharges, and the presence of a strong positive agglutination test in the ewe for 8-10 weeks after abortion (Radostits *et al* 2007).

1.2.4 Toxoplasmosis

Toxoplasmosis is a zoonotic infection of animals caused by the protozoan parasite *Toxoplasma gondii*. It may manifest itself as a disease of pregnancy by multiplying in the placenta and foetus, resulting in abortion or in the birth of weak lambs, which may be accompanied by a mummified foetus. In these cases, the placental intercotyledonary membranes are normal but white foci of necrosis, approximately with 2–3 mm in diameter, may be visible in the cotyledons (OIE 2013).

Infection in aborting animals and pleural fluid of foetus can be studied serologically by modified agglutination test, ELISA (of limited value in adults) and PCR (Radostits *et al.* 2007).

1.3 Mortality in newborn lamb

Neonatal mortality rate is the percentage of lambs that die in the first 28 days of life. A realistic target for neonatal mortality rate in a well-managed flock should be 3% and the upper acceptable limit under any circumstances should be 5%. This rate should be constantly calculated and readjusted throughout the lambing season, in order to monitor any abrupt change (Dennis 1974; Rook *et al.* 1990; Haughey 1991; Bernal 2001).

Disorders of newborn lambs can be broadly classified in two types, based on their aetiology (Dennis 1974; Rook *et al.* 1990; Haughey 1991; Bernal 2001):

- Disorders of non-infectious aetiology;
- Pathological conditions of microbial or parasitic aetiology.

1.3.1 Leading causes of death in Haute Vienne

1.3.1.1 Enterotoxaemia

Enterotoxaemia is more commonly called pulpy kidney and is caused by *Clostridium perfringens* type D (Duncanson 2012), a Gram-positive, rod-shaped, anaerobic, and spore-forming bacteria.

It can be found in growing lambs in commercial flocks that have not been vaccinated adequately (Duncanson 2012). Animals in high state of nutrition on a lush feed, grass or grain, tend to be more susceptible to this disease (Bhat *et al*. 2009).

Lambs from vaccinated mothers should have their first dose of vaccine by 10 weeks of age followed by a second dose 4–6 weeks later. Lamb from unvaccinated mothers should be vaccinated as early as 4 weeks of age, with a second dose 4–6 weeks later. When the risk is very high, a dose of vaccine should be given within the first 2 days of life (Duncanson 2012). Lambs and sheep vaccinated during the previous year require only one injection in the subsequent year (Bhat *et al.* 2009).

The disease is normally manifested by sudden death, although observant keepers can detect sick cold and moribund animals that do not respond to treatment. Necropsy findings are usually sufficient to establish a diagnosis: the abdomen, pleural cavity and pericardium will be filled with fluid, usually bloody (Duncanson 2012); the pulpy texture of the kidney is due to acute tubular epithelial degeneration and/or necrosis and interstitial edema and hemorrhage (McGavin & Zachary 2006) (figure 1). There is also a high level of sugar in the urine, which can be confirmed by a small animal urine dipstick (Duncanson 2012).

A) B) C)

Figure 1: Hemorrhagic kidneys (A and B) of a three week old lamb and a pulpy kidney (C) of four days old lamb with enterotoxaemia. (Maciel 2014)

1.3.1.2 Omphalophlebitis

Umbilical infections (figure 2) may remain localized and develop into a discrete abscess involving the body wall or may extend to peritonitis, urachal infection, liver abscessation and, possibly, more generalized infection involving the joints, meninges, lungs, kidneys and endocardium (Scott 2007).

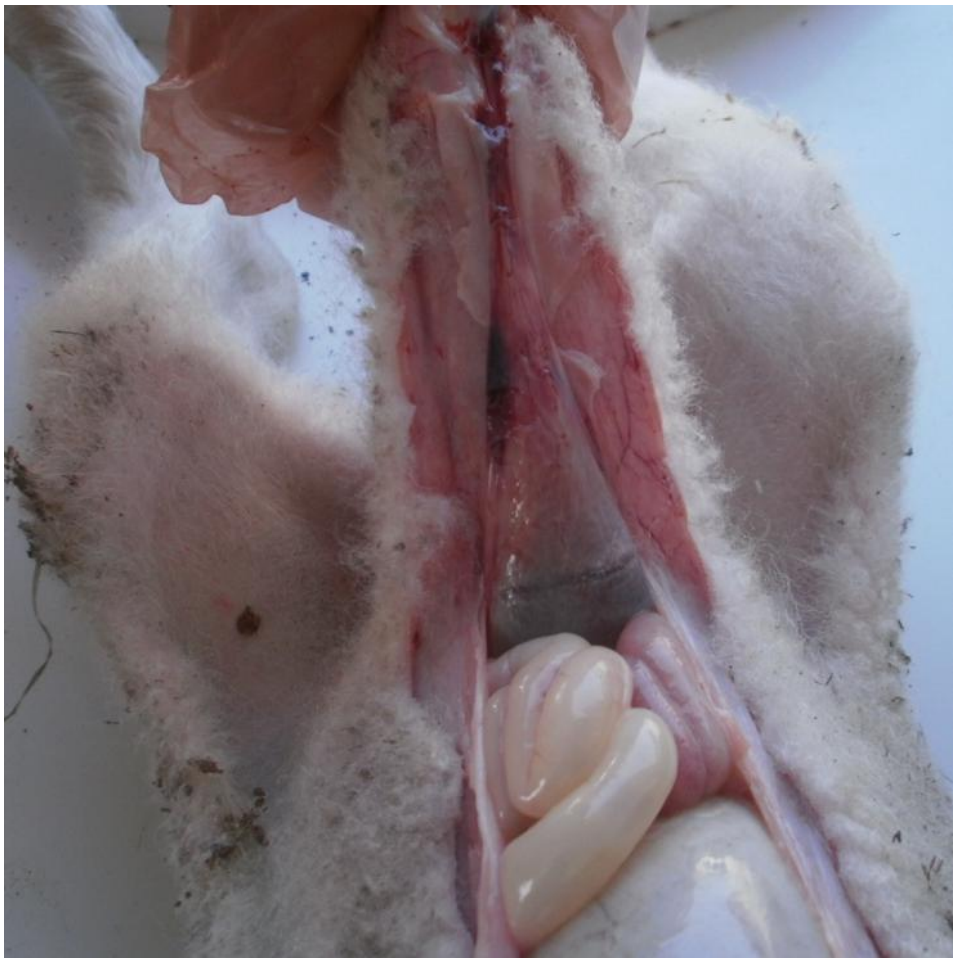


Figure 2: Omphalophlebitis in a four days old lamb with colibacillosis. (Maciel 2014)

The umbilicus must be fully immersed in a highly concentrated iodine solution within the first 15 minutes of life. This must be repeated at least 2–4 hours later. Antibiotic aerosol sprays are much inferior to iodine solutions for dressing navels and, besides that, are much more expensive (Scott 2007).

Failure to adopt sound management practices with respect to hygiene standards and routine navel dressings can lead to considerable lamb mortality (Scott 2007).

1.3.1.3 Colibacillosis

Basically, *Escherichia coli* infections in lambs, also known as watery mouth disease, are largely due to poor management rather than virulent pathogens. The main predisposing factor is likely to be a lack of sufficient good quality colostrum within the first six hours of life (Duncanson 2012). The old adage "a lamb that has had adequate colostrum is hard to kill and a lamb that has not is hard to save", still holds good (Duncanson 2012).

Sick lambs are clearly prostrated, inappetant, hold their heads low and with sialorrhoea. The designation "watery mouth" comes from this last finding. Colibacillosis is also characterized by hypothermia, distended fluid abdomen and decreased ruminal motility. Hypothermia and dehydration often hasten the onset of coma and death. Septicaemia is a constant feature of this disease (Radostits *et al*. 2007) (figure 3).

The condition may be peracute and the death may occur before the lamb is seen with diarrhoea. Isolation of the organism from heart blood usually confirms the diagnostic.

Often unclotted colostrum will be seen in the stomach (Duncanson 2012).

Figure 3: Pericardial effusion in a two days old lamb with colibacillosis. (Maciel 2014)

Regarding antibiotherapy, 40% of these strains of *E. coli* are resistant to ampicillin and a better response is expected with amoxicillin combined with clavulanic acid. Good management, however, is vital and antibiotics should never be used to compensate defective management practices (Duncanson 2012).

1.3.1.4 Coccidiosis

Coccidiosis in lambs is a common disease in housed flocks and can originate diarrhoea and result in death in 1 to 3 weeks. The oocyst output by grazing lamb is very large when compared to the output by ewe. Coccidiosis occurs commonly in lambs following introduction into a feedlot situation, where overcrowding and other stressors are present (Radostits *et al*. 2007).

Lambing grounds should be well drained and kept as dry as possible. All measures that minimize the amount of faecal contamination of hair coats and fleece should be practiced regularly (Radostits *et al.* 2007).

The diagnosis is made by the presence of oocysts in faeces and merozoites in the intestinal tissues.

Addition of decoquinate to mineral salt gave rise to lower oocyst elimination, thus favouring eimeriosis control in sheep (Júnior *et al.* 2012).

Sericea lespedeza, a widely adapted, nonbloating, warm-season perennial legume that can be used for grazing, as hay, or as a conservation plant, was effective in the prevention and control of coccidiosis. Use of *Sericea lespedeza* could reduce lamb losses post-weaning, reduce the need to treat for coccidiosis and create a significant economic benefit to livestock producers (Burke *et al.* 2013). Coccidiostats are used for the control of naturally occurring coccidiosis in lambs (Radostits *et al.* 2007).

2. Materials and methods

2.1. Animals and husbandry

Fifty-seven outdoor flocks managed in extensive breeding systems were studied. This represented approximately 22.614 adult ewes (Mean ± SD: herd size=355.8 ± 255.7 ewes) of the cross-bred between the following races Romanov, Southdown, Suffolk, Texel, Charmoise, Chaurollais, Ile de France, Limousin, Romane and Vendéen distributed throughout the Limousin region. This study was carried out between January and April 2014.

2.2. Necropsy examination

Post-mortem examination of a representative number of neonatal deaths can provide useful information (Scott 2007).

Necropsies of abortions were also performed but in the *Laboratoire Départemental d'Analyses et de Recherche de la Haute-Vienne*.

2.3. Study design and sampling

This was an observational study: the cases of lamb mortality of owners who asked for veterinary support were investigated. The samples were selected using convenience sampling. A cross-sectional analysis of a sample of 249 lambs deaths and 118 ewe abortions from all 57 flocks were registered at Veterinary Clinic *des Rochettes* during the period between January and April of 2014.

The minimum requirements for laboratory submissions for abortion investigation include the foetus(es), foetal stomach content, a piece of placenta and a maternal serum sample. While the first submission may identify a recognized abortifacient agent, it is important to collect aborted material throughout the outbreak, as more than one agent may be present within the flock and such knowledge is essential when planning treatment, control and prevention strategies (Scott 2007).

2.4 Laboratory testing

Sixteen blood samples from sheep that aborted were collected by jugular vein puncture in BD Vacutainer® without additives in the tube (Becton Dickinson, Plymouth, UK). Serum samples were analysed in the *Direction Départementale de la Cohésion Sociale et de la Protection des Populations de la Haute-Vienne* for the detection of *Brucella ovis* (Rose-bengal Plate Test) (Table A-1).

Twenty-seven analyses of foetal and placental tissues from sheep that aborted were processed by the *Laboratoire Départemental d'Analyses et de Recherche de la Haute-Vienne* for the detection of nucleic acid (DNA) of *Chlamydophila abortus* and *Coxiella burnetii* (LSI VetMAX™ Triplex *Coxiella burnetii* & *Chlamydophila spp.* Real-Time PCR Kit, Life Technologies) and *Toxoplasma gondii* (*Toxoplasma gondii* Real-TM, Sacace Biotechnologies) (Table A-1). It also researched *Salmonella* by isolation and identification. The recommendation of this laboratory is to investigate the abortion cause in the following cases:

- If there are 3 abortions in 3 days or less, independently of the size of the breeding herd or lot;
- Abortions spaced over a maximum period of three months by reproduction lot and by lambing period:
    - Less than 250 animals: from 4% of abortions;
    - More than 250 animals: from the 10th abortion.

All cases of coccidiosis were confirmed using the faecal flotation method (Ovatec®, Zoetis).

2.5 Economic losses

Economic losses related with perinatal lamb mortality were crudely estimated basing on the price of lamb indicated by the *Institut de l'Élevage & Confédération Nationale de l'Élevage* (2013).

2.6 Statistical analyses

The statistical package used for all statistical analyses was the Microsoft Office® Excel 2007 and IBM® SPSS® Statistics 22.

Frequencies of clinical status before death and necropsy findings were compared using the non-parametric test Fishers instead of Pearson chi-square test because the frequencies into the groups are very small, as only 20% of the cells present expected count upper than 5.

Flocks were stratified in six groups according to the adult ewe number for a more careful analysis of abortions: Group 1: [40-200] adult ewes; Group 2:]200-400] adult ewes; Group 3:]400-600] adult ewes; Group 4:]600-800] adult ewes; Group 5:]800-1000] adult ewes and Group 6:]1000-1200] adult ewes. Since Group 1 was a flock with a ratio of abortions/ewe (flock number 5, Table A-2) much bigger than the others due to an unknown cause, it was removed from this study in order to avoid skewness in the results.

Flocks were also stratified in six groups, according to the adult ewe number, for lamb mortality analysis: Group 1: [4-200] adult ewes; Group 2:]200-400] adult ewes; Group 3:]400-600] adult ewes; Group 4:]600-800] adult ewes; Group 5:]800-1000] adult ewes and Group 6:]1000-1200] adult ewes.

Specific age groups analysed were the following: under 15; [15–30] and [31–45] days old.

Necropsies were stratified in three groups according to the owner's report: group 1 - lambs with diarrhoea before death, group 2 - lambs with sialorrhoea before death and group 3 - lambs that died suddenly, without observable symptoms.

3 Results

3.1 Prenatal mortality

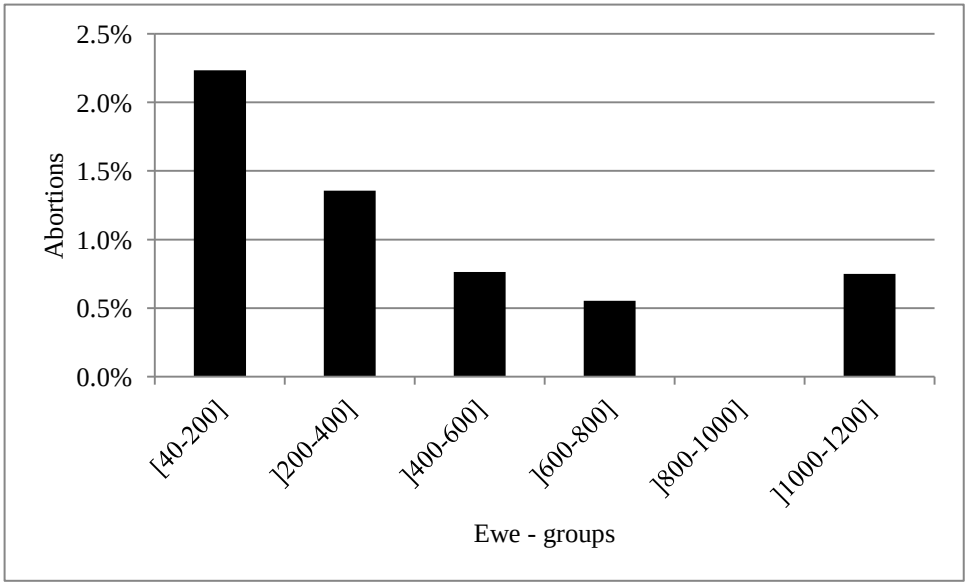
Although the study had started in January, the author considered interesting to analyse data from September onwards because, considering the five months of pregnancy, the lambing would occur during this study.

Of the 57 herds tested with approximately 22.614 adult ewes (Mean ± SD: herd size=355.81 ± 255.68 ewes), abortions occurred in 26, making up a total of 118 abortions (Mean ± SD: lambs abortions *per* affected herd=4.54 ± 4.47), from September 2013 to April 2014 (Table A-1).

As the CV=98.46% was greater than 50%, it indicated a very high dispersion of values and consequently we decided to analyse the percentage of abortions in the sheep farms in groups. For a more careful analysis according to the adult ewe number, we draw the graphic 1 based in the table 1.

	Number of flocks	Adult ewe	Adult ewe/flock	Abortion number	Abortion/flock %
	8	940	[40-200]	21	2.23
	5	1550	]200-400]	21	1.35
	5	2750	]400-600]	21	0.76
	6	4700	]600-800]	26	0.55
	0	0	]800-1000]	0	0.00
	1	1200	]1000-1200]	9	0.75
Total	**25**	**11140**	**-**	**98**	**-**

Table 1: Percentage of abortions into six groups according adult ewe/flock.



Graphic 1: Percentage of abortions into six groups according adult ewe/flock.

From those 26 herds which had abortions, only 17 (65%) sent foetuses, placenta or blood to the laboratory. Of those 17 herds which sent samples to the laboratory, it was not possible to identify any etiologic agent in 10 herds (59%). The etiologic agents are represented briefly in table 2 but can be consulted detailed in Table A-2:

	Tested adul ewe	Total positive ewe
Brucella ovis	10	0 (0%)
Coxiella Burnetti	26	3 (12%)
Chlamydophila spp.	20	2 (10%)
Toxoplasma gondii	18	2 (11%)
Gram stain	2	2 (100%)
Salmonella spp.	17	2 (12%)
Listeria ivanovii	1	1 (100%)

Table 2: Abortion etiologic agent.

3.2 Lamb mortality

Between January and April 2014, 47 lambs were necropsied at the Veterinary Clinic *des Rochettes* or *in-situ* on the farms. The age of the lambs ranged from 2 to 45 days old.

Necropsies were stratified in three groups according to the owner's report: group 1 - 18 (38.3%), lambs with diarrhoea before death; group 2 - 3 (6.4%), lambs with sialorrhoea before death; and group 3 - 26 (55.3%), lambs that died suddenly. The crude mortality rate of lambs in four months was 1.41% (Table A- 3).

The presumptive diagnosis of the necropsied lambs is represented in table 3. In the group "other" the following cases were identified: suspected case of *Schmallenberg* virus infection (1), maternal-fetal disproportion (2), acidosis (3), helminthosis (1), starvation (3), enteritis (1), cryptosporidium (2) and ecthyma (1).

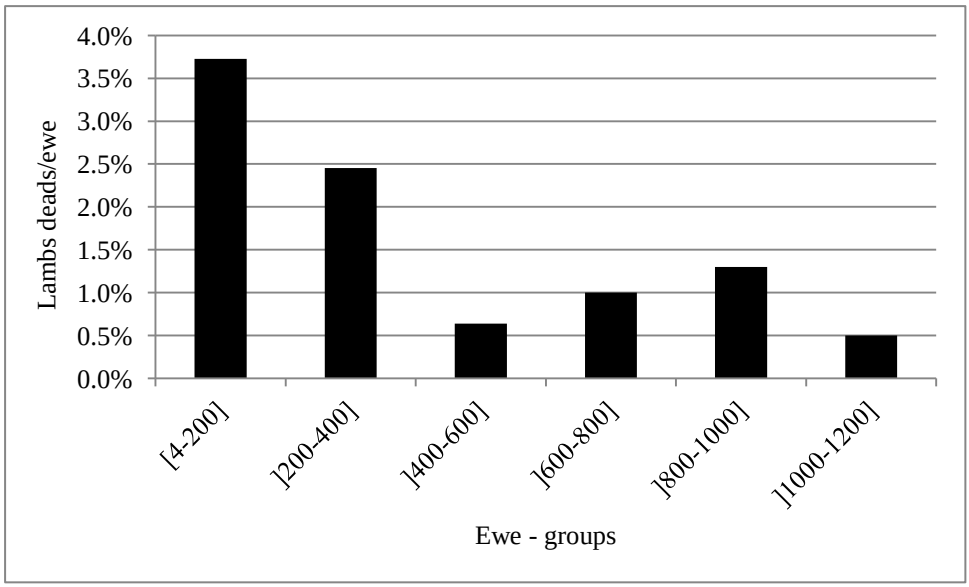
	Frequency	Percent
Colibacillosis	7	14.9
Enterotoxemia	12	25.5
Coccidiosis	4	8.5
Septicemia	9	19.1
Other	15	31.9
Total	**47**	**100.0**

Table 3: Presumptive diagnosis of the necropsied lambs.

Producers also informed the Veterinary Clinic *des Rochettes* of over 202 dead lambs, which together with the 47 lambs necropsied, accounted for a total of 249. The distribution of deaths by groups of flocks is represented in table 4 and graphic 2.

	Number of flocks	Adult ewe	Ewe - groups	Lambs deads	Lambs deads/ewe
	19	2174	[4-200]	81	3.73%
	11	3300	]200-400]	81	2.45%
	9	5000	]400-600]	32	0.64%
	4	3000	]600-800]	30	1.00%
	1	1000	]800-1000]	13	1.30%
	2	2400	]1000-1200]	12	0.50%
Total	**46**	**16874**	-	**249**	-

Table 4: Distribution of deaths by groups of flocks.



Graphic 2: Distribution of deaths by groups of flocks.

3.2.1 The age-cause proxy relationship in lamb mortality

By necropsy group

In table 5, it is possible to analyse the relationship between clinical status before death and the age of the dead lamb.

	Age groups							
	Group 1: 0-14		Group 2: 15-30		Group 3: 31-45		Total	
	Count	%	Count	%	Count	%	Count	%
Diarrhoea	5	27.8	4	22.2	9	50.0	18	38.3
Sialorrhoea	1	33.3	1	33.3	1	33.3	3	6.4
Without observable symptoms	11	42.3	8	30.8	7	26.9	26	55.3
Total	**17**	**36.2**	**13**	**27.7**	**17**	**36.2**	**47**	**100.0**

Table 5: Relationship between clinical status before death and the age of the dead lamb.

By post-mortem diagnosis

In table 6, it is possible to analyse the relationship between disease and the age of the deceased lamb.

		Disease										
		Colibacilosis		Enterotoxemia		Coccidiosis		Septicemia		Others		Total
		Count	%	Count	%	Count	%	Count	%	Count	%	Count
Age groups	0-14	2	11.8	3	17.6	1	5.9	7	41.2	4	23.5	17
	15-30	1	7.7	5	38.5	1	7.7	2	15.4	4	30.8	13
	31-45	4	23.5	4	23.5	2	11.8	0	0.0	7	41.2	17
	Total	**7**	**14.9**	**12**	**25.5**	**4**	**8.5**	**9**	**19.1**	**15**	**31.9**	**47**

Table 6: Relationship between disease and the age of the deceased lamb.

3.2.2 Integrating animal alive and necropsy findings

We could observe the following after analysing the results of the necropsy:

- Six septicaemia cases were detected at the necropsy. In 5 cases (83.3%), omphalophlebitis was present/observed/detected;
- Six colibacillosis cases were detected. In 5 of these cases (83.3%), liver congestion was present; in 3 cases (50%), pericardial congestion; in 4 cases (66.6%), omphalophlebitis; and in 2 cases (33.3%), kidney congestion;
- Four coccidiosis cases were detected at the necropsies. In 2 cases (50%), pulmonar hepatization was present/observed while it was observed nephritis other 2 cases (50%);
- Nine enterotoxemia cases were detected at the necropsied. In 4 cases (44.4%), pericarditis was detected, liver congestion in 2 cases (22.2%), pulpy kidney in 5 cases (55.5%) and mesenteric lymphadenitis in 3 cases (33.3%).

In table 7, it is possible to analyse the association between diarrhoea and the following diseases: colibacillosis, enterotoxemia, and coccidiosis.

			Diarrhoea		
			No	**Yes**	**Total**
Disease	**Colibacillosis**	Frequency	**5**	**2**	**7**
		With disease	71.4%	28.6%	100.0%
	Enterotoxemia	Frequency	**7**	**3**	**10**
		With disease	70.0%	30.0%	100.0%
	Coccidiosis	Frequency	**0**	**4**	**4**
		With disease	0.0%	100.0%	100.0%

Table 7: Association between diarrhoea and the following diseases: colibacillosis, enterotoxaemia, and coccidiosis.

As diarrhoea was reported in 38.3% of the necropsied lambs, we analysed if in these necropsies, the proportion of cases with diarrhoea was significantly different from cases without diarrhoea, i.e. if the proportion of cases of diarrhoea is significantly different from 0.5:

- Binomial test showed:
  - 95% CI 0.24-0.63; $P = 0.66$

3.3 Economic losses

The total number of lambs that died was 367: 118 aborts and 249 natural causes (Mean ± SD: lambs loss *per* herd=6.1 ± 8.0 lambs) in a total of 57 herds analysed in four months, therefore 1101 animals in a year (table A-4), assuming that mortality is constant throughout the year, which does not happen.

The price of a lamb in Limousin region is actually around 106€. Although the price of a lamb at slaughter is not equal to the price of a weakened animal or an abortion, one can plausibly estimate a total annual loss of 116706€ (367animals x 106€ x 3).

Since the annual ewe prolificacy is 1.05 lambs/ewe/year, in the fifty-seven outdoor flocks studied with approximately a total of 22.614 adult ewe, it would be expected the lambing of 23.744. With the numbers of abortions and deaths already mentioned, the actual number of live lambs would be closer to 22.643, assuming that there are 1101 animals deaths in a year (table A-4).

Thus, there is an average animal loss rate in Limousin region of 13.05% (Table A-4), assuming that mortality is constant throughout the year, which does not happen.

4 Discussion

First of all, it is important to emphasize that the sample was selected using convenience sampling. In this study, we investigated the cases of lamb mortality of owners who asked for veterinary support so, when convenience is the main criterion for selecting a sample, it is very unlikely that the sample will be truly representative of the study population, resulting in biased estimates (Thrusfield 2007). Ideally, all clients of the clinic should have been contacted to know the number of abortions and dead lambs. Thus, the rate of abortions and neonatal mortality would truly represent the reality of farms which Veterinary Clinic *des Rochettes* supports. Another important note relates to the fact that the diagnoses are merely presumptive so it should only be read as a guidance and not as a confirmation of the causes that lead to death in lambs in Limousin region.

The analysis of the graphics 1 and 2 permits to conclude that, in general, farms with fewer ewes have higher lamb mortality. The author recommends further investigation regarding familiar management and professional management to explain these cases.

According to table 5, it is possible to conclude that diarrhoea affected more lambs with 31-45 days old (50%). Sialorrhoea achieved a homogeneous distribution across age groups.

According to tables 5 and 6, the majority of lambs (55%) died without showing any clinical signs and lambs with 0-14 and 31-45 days old are those who die more (36%).

4.1 Abortion

The prenatal mortality, represented in Table A-1, presents the agents detected in reviews of foetuses and placentas but it does not mean that the cause of death was the presence of this agent in all these cases. Sometimes the state of decomposition of the foetus and the contact with the ground results in the contamination with ubiquitous agents responsible for positive results, obviously not necessarily involved in the death. Thus, the author recommends caution when formulating hypothesis based on foetal or placental analyses. Ideally, these results should be considered in association to the mother's blood analyses (Table A-2).

Unfortunately, the results of this study indicate that it was not possible to identify the ethiologic agent in most of the herds animals (59%). The fact that most producers do not have a rationale answer for the cause of abortion is in accordance with the study of Kirkbride (2012), that shows that it was not possible to identify a cause in 56% of the ovine abortions. This fact helps to explain the reason why many farmers do not usually send samples to the laboratory. Due to the small positive agent sample size, it is impossible to define the leading abortion causes. The use of methods for the detection of other agents in Limousin region should also be considered.

As an abortion rate above 2% suggests an infectious aetiology, laboratory investigation is strongly recommended (Scott 2007). The analysis of the graphic 1 permits to conclude that the smaller flocks, [40-200] adult ewes, have abortion rate above 2% in opposition to the bigger flocks. Familiar and professional management practices can be the answer for this difference but further investigation should be addressed to explain this.

4.2 Post-mortem diagnosis

As Yen and others (2007) refer, one limitation of the necropsy is that it is subjective and observer-dependent (Yen *et al.* 2007).

4.2.1 Enterotoxaemia

Although the toxoid is included in all the vaccines (Duncanson 2012), enterotoxaemia was the leading cause to death of all necropsied lambs (25.5%). Above all, it is important to raise awareness of farmers by informing them about the importance of lambs and ewe vaccination. Vaccinating ewe and lambs against enterotoxaemia is a very viable option for saving money. Only for information, in the Limousin region it is possible to purchase a dose/animal by 0.37€ (Bravoxin 10® 100ml, MSD Animal Health) or 0.60€ (Coglavax® 50ml, Ceva).

It is interesting to note that in the cases of enterotoxemia, 22.2% showed liver congestion (figure 4). Epsilon (ε) toxin promotes liver glycolysis (Pugh & Baird 2012) as they act directly on the liver, which explains the hepatic inflammation and the subsequent congestion. In this study, 44.4% of enterotoxaemia cases showed pericarditis and 33.3% showed mesenteric lymphadenitis. Although this disease implies for many people a "pulpy kidney" (figure 5), only 55% of cases showed the presence of kidneys with altered consistency. Enterotoxemia affects more lambs with 15-30 days old (table 6). According Scott (2007) is more common in four days old lambs.

Figure 4: Liver congestion. (Maciel, 2014)

Figure 5: Pulpy kidney of a three days old lamb. (Maciel, 2014)

4.2.2 Colibacillosis

The colibacillosis, cause to death of 14.9% lambs necropsied, can be explained in a similar way to that of the enterotoxaemia: liver, pericardial and kidney congestion mean systemic infection and omphalophlebitis is one of the gateways to the *E. coli*.

Although veterinary use of colistin has not been proven to increase the resistance to colistin in humans, its utilisation must be carefully pondered. The European Medicines Agency recommends maintaining the use of colistin in veterinary medicine but restricting its use to the treatment of infected animals and those in contact with them, and to remove all indications for prophylactic use. Thereby, the author considers that its veterinary use should be responsible because colistin is reserved for use in multi-drug resistant infections in humans.

Some authors suggest the substitution of colistin for veterinary use by gentamicin since it is not as critical with respect to use in humans and to save colistin as the last resource for clinical isolate of Extended-Spectrum Beta Lactamase and resistant to carbapenems (World Health Organization 2011, Bariagye R. *et al.* 2012, and Medina *et al.* 2011). On the other hand, to complete this reasoning, Radostits *et al.* (2007) advises the joint use of gentamicin with penicillin. Amikacin sulfate may also be an option in the view of the author with regard to its excellent Gram-negative activity (Radostits *et al.* 2007) and its classification in the priorization within critically important antibiotics of World Health Organization (2011).

Colibacillosis affects more lambs with 31-45 days old (table 6). Scott (2007) refers that colibacillosis is more common in orphan lambs but we didn't confirm that information in this study.

4.2.3 Omphalophlebitis

Despite the fact that the number of samples tested was rather limited, the comparative analyses gives clear indications that omphalophlebitis (figure 6) increased the lambs' risk for developing sepsis. In our study, sepsis was the cause to death of 19.1% lambs necropsied and omphalophlebitis was present in 83.3%. Navel disinfection with povidone iodine (7% tincture), a weak iodine solution or a chlorhexidine solution is recommended. The chlorhexidine solution appears to have a more prolonged residual antibacterial effect, while the strong iodine solutions may be associated with formation of umbilical abscesses or persistent urachus (Pugh & Baird 2012). However, ligation of umbilical vessels at the level of the abdomen using plastic clamps, available for this purpose, may be more effective (Radostits *et al.* 2007).

Figure 6: Omphalophlebitis in a day old lamb. (Maciel, 2014)

The risk of septicaemia, arthritis and fever originating in a navel infection, can be exacerbated by the failure of transfer of passive immunity (Larson *et al.* 2004), emphasizing the need of a proper intake of colostrum to the newborn.

In general, broad-spectrum antimicrobials are chosen because it is almost impossible to predict, based on clinical signs, the nature of the infecting agent and its antimicrobial susceptibility. Due of the wide variety of infecting agents and their varying antimicrobial susceptibility, it is possible to make only general recommendations for antimicrobial therapy of neonates (Radostits *et al.* 2007).

Septicemia affected more lambs with 0-14 days old (table 6), which is in agreement with Scott (2007).

4.2.4 Coccidiosis

Although all animals with coccidiosis had diarrhoea prior to death (table 7), more positive cases may have been overlooked as acute coccidiosis can be difficult to diagnose due to the fact that death can occur before oocysts appear in the faeces, approximately 2 weeks after infection (Pugh & Baird 2012). In 2 cases (50%), pulmonar hepatization was observed, while nephritis was present in 2 (50%) cases. As it was not possible to establish a cause-effect, further investigation should be addressed.

Cocidiosis affected more lambs with 31-45 days old (table 6), which is in agreement with Radostits *et al*. (2007), who refers that coccidiosis occurs commonly in lambs following introduction into a feedlot situation, where overcrowding and other stressors are present.

4.2.5 Starvation / hypothermia / hypoglycaemia

Although starvation/hypothermia/hypoglycaemia has not been a major cause of lambs death, the producers reported this death. It was found that many producers devaluated sporadically death of lambs and were not interested in investigating its cause. It is remarkable that the keepers only care about multiple consecutive deaths. In a time of the year when the temperature reaches -3ºC in many days, adding to the fact that many barns had an ineffective ventilation with drafts, the absence of any hypothermia diagnose case is, at least, suspect or questionable.

It was decided to examine the relation between diarrhoea and the following diseases: colibacillosis, enterotoxaemia or coccidiosis. This point could be helpful to establish a prophylactic protocol in a problematic flock but like table 7 shows, it is difficult to achieve a really definitive conclusion in that way. Only 30% of lambs with colibacillosis or enterotoxaemia had diarrhoea before death, so we can not say that all lambs with these diseases got diarrhoea before death. However, and in the case of coccidiosis, it is correct to affirm that 100% of lambs with this disease presented diarrhoea before death.

The binomial test showed that the proportion of cases with diarrhoea is not significantly different from cases without diarrhoea (95% CI 0.24-0.63; $P = 0.66$).

4.3 Economic losses

The analysis of the graphic 1 permits to conclude that the smaller flocks, [40-200] adult ewes, have a higher abortion rate (above 2%) than the bigger flocks.

The calculation of the total annual losses intended to show without too much preciousness, the possible economical impact on the farms. In order to be more precise, this calculation should discount the price of the food that the animal would eat to be ready for slaughter, which reduces the economic losses due to an early death.

5 Conclusion

The problem of perinatal lamb mortality is very important, both from the viewpoint of animal welfare and farm economy. Therefore, it is important that veterinarians continue to cooperate with farmers to create the conditions in order to reduce perinatal lamb mortality.

Many of the predisposing factors and causes of lamb mortality can be avoided or reduced by adopting simple and low-cost measures, like raising the producer awareness to the importance of cattle vaccinating against enterotoxaemia (disease which caused a lamb death of 25.5%) doing routine coprology to deworm before parasitism becomes a parasitic disease (remember that coccidiosis caused 8.1% of lamb death), and adopting aseptic care of the newborn umbilical cord (83% of septicemia cases and 67% of colibacillosis cases were caused by omphalophlebitis).

It is also important to isolate the involved agents and subsequently determine their drug susceptibility as well as other equally effective therapeutic solutions. Therapies with lower potential effects on human health must be introduced in order to reduce the risks of the emergence of multidrug-resistant agents. The first step to reduce the use of antibiotics and other treatments is the elimination of the environmental conditions that favour the emergence of health problems.

The results of this study indicate that it was not possible to identify the etiologic agent in most of the herds (59%). Further investigation should be done to study new methods to explore abortion etiologic agents and to start to select other agents in the Limousin region to increase the farmers confidence in these results tests.

One should always bear in mind that animal production is a business and is vital to the livelihood of many families. More important than treating, the main purpose of the veterinarian should be a closely monitoring of the day-to-day operations of each farm to be able to detect and prevent many potential health problems that may. As the mortality rate was higher in lambs with with 0-14 and 31-45 days old (36%) and as the majority of lambs (55%) died without showing any clinical signs, one should conclude that it is important to invest in prevention. Ideally, a veterinarian should integrate all information resulting from the anamnesis, autopsy findings, biochemical parameters, routine coprology, evaluation of infrastructures, farm economy, etc. Only then we can contribute to the increase of the farmers profits, who will in turn regard veterinarians not as an expense but as a cornerstone in their business development.

6 Conflict of interest statement

The author affirms that there are no conflicting interests involved in the production of this dissertation.

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Appendix-Datas

Flock	Adult ewe	Abortions	Number abortions/ewe per flock
1	40	1	2.50%
2	50	2	4.00%
3	50	3	6.00%
4	50	1	2.00%
5	50	20	40.00%
6	150	2	1.33%
7	200	6	3.00%
8	200	5	2.50%
9	200	1	0.50%
10	250	1	0.40%
11	300	10	3.33%
12	300	4	1.33%
13	300	1	0.33%
14	400	5	1.25%
15	450	7	1.56%
16	500	7	1.40%
17	600	2	0.33%
18	600	1	0.17%
19	600	4	0.67%
20	700	10	1.43%
21	800	1	0.13%
22	800	2	0.25%
23	800	2	0.25%
24	800	1	0.13%
25	800	10	1.25%
26	1200	9	0.75%

Table A-1: Relationship between herd size and frequency of abortions

Flock	Adult ewe	Abortions	Sample	*Brucella ovis*	*Coxiella Burnetti*	*Chlamydophila* Spp	*Toxoplasma gondii*	Gram Stain	*Salmonella*	Bacteriological test
1	400	ND	fetal and placental tissues		✘	✘	✘		✘	
2	700	10	serum ewe	✘						
			fetal tissue		✘	✘	✘		✘	
3	200	6	fetal tissue		✘	✘	✘		✘	
4	800	2	2 fetal tissue		✘	✘	✘		✘	
5	800	2	placental tissue				✘		✘	
			fetal tissue		✓	✓				
			fetal tissue		✘	✓				
			serum ewe	✘						
			serum ewe	✘						
6	200	5	serum ewe	✘	✘	✘	✓		✘	
			fetal tissue		✘	✘	✘		✘	
7	500	ND	fetal tissue		✘	✘	✘			
			fetal tissue		✘	✘	✘			
			placental tissue		✓	✘				
8	300	4	fetal tissue		✘	✘	✘		✘	
			2 fetal tissue		✓		✘		✘	
9	900	ND	2 fetal tissue		✘	✘	✘	Gram+ rods	*Salmonella enterica* subsp. arizonae	*Listeria ivanovii*
10	1,200	9	serum ewe	✘						
			2 fetal tissue		✘	✘			✘	
11	600	2	fetal tissue		✘	✘	✘		✘	
			serum ewe	✘						
			serum ewe	✘						
			3 fetal tissue		✘	✘	✘		✘	
12	50	1	fetal tissue		✘	✘	✘		✘	
13	150	2	serum ewe		✘					
			serum ewe		✘					
			serum ewe		✘					
			serum ewe		✘					
			serum ewe		✘					
14	800	10	serum ewe	✘						
			fetal tissue		✘	✘	✘		✘	
15	50	20	serum ewe	✘						
			fetal tissue		✘	✘	✓		✘	
16	600	4	serum ewe	✘						
17	400	5	2 fetal tissue		✘	✘	✘	Gram- rods	*Salmonella abortusovis*	

Table A-2: Results of abortions laboratory analyses (✘: negative result ;✓: positive result).

Adult ewe	Lambs dead	Lambs necropsy	Expected number of lambs/year/farm	Rate mortality/farm
4	1	-	4	25.00%
50	10	-	52	19.23%
50	5	1	52	9.62%
50	1	-	52	1.92%
50	3	3	52	5.77%
50	15	1	52	28.85%
50	14	3	52	26.92%
70	4	2	73	5.48%
100	1	1	105	0.95%
100	3	2	105	2.86%
150	2	-	157	1.27%
150	2	-	157	1.27%
150	2	-	157	1.27%
150	2	2	157	1.27%
200	5	-	210	2.38%
200	1	-	210	0.48%
200	1	-	210	0.48%
200	8	2	210	3.81%
200	1	-	210	0.48%
250	1	-	262	0.38%
250	6	4	262	2.29%
300	1	-	315	0.32%
300	2	-	315	0.63%
300	6	-	315	1.90%
300	2	-	315	0.63%
300	3	-	315	0.95%
300	2	1	315	0.63%
300	8	2	315	2.54%
300	48	-	315	15.24%
400	2	-	420	0.48%
500	3	-	525	0.57%
500	5	1	525	0.95%
500	2	-	525	0.38%
500	6	1	525	1.14%
600	3	2	630	0.48%
600	1	-	630	0.16%
600	4	-	630	0.63%
600	3	-	630	0.48%
600	5	2	630	0.79%
700	13	4	735	1.77%
700	7	3	735	0.95%
800	5	2	840	0.60%
800	5	2	840	0.60%
1000	13	4	1050	1.24%
1200	7	-	1260	0.56%
1200	5	2	1260	0.40%
Total	**249**	**47**	**17711**	

Table A-3: Mortality of newborn lambs in four months

Abortions and Lambs dead	Expected number of lambs/year/farm	Mortality rate/farm
1	315.0	0.32%
1	840.0	0.12%
1	105.0	0.95%
1	262.5	0.38%
1	210.0	0.48%
1	210.0	0.48%
1	42.0	2.38%
1	4.2.0	23.81%
2	157.5	1.27%
2	315.0	0.63%
2	840.0	0.24%
2	157.5	1.27%
2	525.0	0.38%
2	840.0	0.24%
2	210.0	0.95%
2	52.5	3.81%
2	630.0	0.32%
2	630.0	0.32%
2	315.0	0.63%
2	157.5	1.27%
3	525.0	0.57%
3	630.0	0.48%
3	315.0	0.95%
3	630.0	0.48%
3	105.0	2.86%
4	52.5	7.62%
4	52.5	7.62%
4	157.5	2.54%

Abortions and Lambs dead	Expected number of lambs/year/farm	Mortality rate/farm
4	73.5	5.44%
5	52.5	9.52%
5	525.0	0.95%
5	210.0	2.38%
5	840.0	0.60%
5	630.0	0.79%
5	1260.0	0.40%
6	840.0	0.71%
6	315.0	1.90%
6	525.0	1.14%
7	525.0	1.33%
7	472.5	1.48%
7	420.0	1.67%
7	262.5	2.67%
7	735.0	0.95%
8	210.0	3.81%
8	315.0	2.54%
8	630.0	1.27%
10	52.5	19.05%
10	735.0	1.36%
10	840.0	1.19%
11	210.0	5.24%
13	1050.0	1.24%
13	735.0	1.77%
15	52.5	28.57%
16	315.0	5.08%
16	1260.0	1.27%
34	52.5	64.76%
49	315.0	15.56%

Table A-4: Mortality and abortions rate obtained in four months